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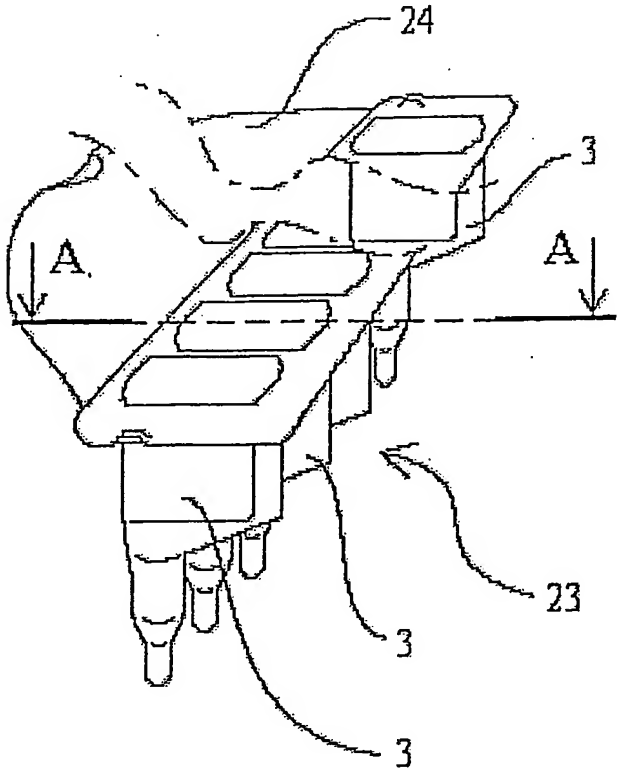
(54) Title: DEVICE AND METHOD FOR SEPARATING, MIXING AND CONCENTRATING MAGNETIC PARTICLES WITH A FLUID AND USE THEREOF IN PURIFICATION METHODS



(57) Abstract: The present invention is related to a process for manipulating magnetic particles that are suspended in a fluid, possibly containing a biological entity of interest, the magnetic particles being able to bind the entity of interest, the fluid being contained in a reaction vessel constituted by a large upper compartment with a funnel shape, an elongate lower compartment with a substantially constant cross-section and a closed base. The process consists of: a) subjecting the magnetic particles to two magnetic fields applied simultaneously to separate all said magnetic particles present in at least the upper compartment of the vessel from the fluid, b) transferring the separated magnetic particles from the upper compartment to the elongated lower compartment, c) removing the fluid from the vessel, d) adding a washing liquid to the lower compartment), e) subjecting the rest of the fluid to at least two magnetic fields applied successively with different and changing directions to wash all the magnetic particles present in the lower compartment, and concentrating said magnetic particles in said lower compartment. The according to the present invention are especially useful in methods for the extraction of nucleic acid to enable them for further processing.

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Device and method for separating, mixing and concentrating magnetic particles with a fluid and use thereof in purification methods

This invention relates to the use of magnetic or magnetizable particles, and, in particular, to methods of separating, mixing and concentrating magnetic or (super) paramagnetic particles efficiently with a fluid and optionally followed by resuspension of the particles in another fluid. The invention further provided a device for doing the same. In the below-exposed invention, the magnetic particles, paramagnetic particles and superparamagnetic particles will be called magnetic particles.

State of the art concerning the separation step:

In many methods of biological analysis, a solid phase has to be separated from a liquid phase and subsequently washed. To wash the solid phase, a defined amount of buffer solution is pipetted into the reaction vessel containing the solid phase to suspend the solid phase in the buffer solution. The solid and the liquid phases are then separated. The liquid phase is then removed by suction (aspiration) and a new washing process begins. Usually a number of washing cycles are carried out, each including a suspension, separation and aspiration process.

The use of magnetic particles as a solid phase and separation by permanent magnets is known in principle. Permanent magnets attract the particles to the wall of the reaction vessel and hold them there.

Magnetic particles are often used in separation processes. There are many biological assay methods and purification methods in which magnetic particles are used. For example, immunoassay methods, nucleic acid hybridisation assays and the like. Magnetic particles can also be used in purification methods, to isolate particular components, proteins, nucleic acids, from the material in which they were contained. The particles can be used to separate certain components from a mixture, for example, because they are coated with a reagent with a specific affinity for the component. Magnetic particles can be drawn to, for example, the wall of a container in which the fluid with the magnetic particles was contained and the fluid can be removed and, optionally, be replaced with another fluid. Thus, the particles can be mixed with the fluid from which the specific

component is to be removed, the component will bind to the magnetic particle, and a magnet can be used to separate the particles with the component from the remainder of the mixture in the fluid. Optionally the magnetic particles can be washed, and can be separated in another fluid. Or the component can be removed from the particles again into another fluid.

European patent application EP-A-0.136.126 describes a device for separation during solid-phase immunoassays. The bottom end of a reaction vessel containing magnetic particles is disposed between two permanent magnets. The axes of magnetization are at right angles to the wall of the reaction vessel, thus reducing stray magnetic fields.

International application WO-A-92/05443 describes a device for separating magnetic particles. The reaction vessels containing the magnetic particles are disposed in rows. Between the rows is positioned a magnetic block. The reaction vessels are disposed in the magnetic block such that two magnets-are diametrically opposite relative to the reaction vessel. The magnets have alternating polarities and their magnetization axes extend parallel. Separated particles are on only one side of the reaction vessel.

The US-A-4,895,650 patent, the contents of which is herein incorporated by reference, describes a separating device in which particles are separated by a permanent magnet. The magnet is on only one side of the reaction vessel. The relation between the level of test solution in the test-tube and the position of the magnet is focused on. The position of the magnet, more particularly its height, must coincide with the level of test solution in the reaction vessel, and is brought to the desired height by packing material in the bottom part of the device holding the magnet.

During an immunoassay, the fluid level in the reaction vessels after adding the required reagents is not necessarily uniform. For example, the level in the reaction vessel after adding conjugate solution may be lower than the level after adding washing buffer solution. The method of analysis described in this former US-A-4,895,650 patent does not take these differences in level into account.

Known devices for separating magnetic particles have the disadvantage of requiring a relatively long time before all magnetic particles are separated from the liquid phase. Separation time may be considerable, particularly for larger volumes.

A device for rapid separation of magnetic particles is described in European patent application EP-A-0.317.286. In this device, the reaction vessel is surrounded by four permanent

magnets (magnets 1, 2, 3 and 4), which are uniformly distributed around a reaction vessel. The direction of the magnetic field of magnets 1 and 3 is rotated through 180° relative to the direction of the magnetic field of magnets 2 and 4. This device has the disadvantage of requiring a relatively large number of permanent magnets to speed up separation. It also excludes many possible cell movements.

In the EP-B-0.644.425 patent is presented an analyser having a device for separating magnetic particles from a suspension, the separation device containing two permanent magnets between which the reaction vessel containing a suspension is located. For faster and more complete separation of the magnetic microparticles, the magnets are located diametrically opposite with respect to the reaction vessel and the pole axes of the magnets form an acute angle with the longitudinal axis of the reaction vessel. An aim of this invention is to provide an analytical device comprising a device for separating magnetic particles such that the magnetic particles in suspension can be rapidly separated even when the reaction vessel is filled to different levels. Another aim is to provide an analytical device for separating magnetic particles such that the magnetic particles in suspension can be separated in a focused manner.

If these documents can be considered for the separation of magnetic particles from a liquid of interest, they cannot authorize a performant mixing that could efficiently wash the particles and give them all chance to bind onto the magnetic particle's surfaces. This efficient step of mixing is absolutely necessary for purifying nucleic acid targets from a biological sample.

State of the art concerning the mixing step:

Purification methods for nucleic acid using magnetic particles have for example been described in various applications such as EP-A-0.757.106 and WO-A-96/41811. In these applications methods are described wherein a sample solution containing nucleic acids is treated with a chaotropic substance to release the nucleic acid. After releasing the nucleic acids from the biological entity in the lysis buffer, the nucleic acids are bound to the magnetic particles. Both particles coated with a target-specific probe as well as particles having a metal oxide coating (e.g. silica), giving a generic binding of all nucleic acids contained in the sample are used for this purpose. After binding the target, interfering components such as cell debris, enzymes, proteins

anti-coagulants and salt are removed by washing the magnetic particles in a (set of) wash buffer(s). Finally, the purified nucleic acids are released from the particles by mixing the particles in a small volume of elution buffer.

For efficient washing and elution the magnetic particles need to be well dispersed and mixed in the relevant buffers. In general, this washing and elution process may be hampered by the aggregation or clogging of the magnetic particles either caused by the adsorption of specific components in the lysed sample (e.g. genomic DNA) or by residual magnetic dipole fields induced in the particles. In particular, the use of silica coated (magnetic) particles with samples that contain significant amounts of genomic DNA (whole blood, sputum, tissue), results in a tight pellet that is difficult to process.

Well-known methods for mixing (magnetic) beads in a liquid buffer are vortexing, sonification or pipetting. These methods however are difficult to automate, and/or give risk of sample to sample contamination by aerosol generation or they may degrade the nucleic acid target. Furthermore, these methods are not well suited for very small volumes of liquid (typically 0.01ml) as may be required for the elution process.

The method and device according to the invention are especially suitable for use with isolation procedures, where, usually an ingredient is to be isolated in rather pure form from a relatively large volume of sample fluid, and concentrated into a smaller volume of another fluid to be suitable for further use.

In the case of a method for the isolation of nucleic acid such further use may be a nucleic acid amplification method or an assay for the detection of nucleic acid or both.

A method and apparatus for separating and resuspending superparamagnetic particles is disclosed in the application WO-A-91/09308. In this application it was disclosed that superparamagnetic particles may be aggregated and resuspended by subsequent application of different magnetic fields. First and second applications of the magnetic field could be provided with the same magnet, which was then rotated around the container containing the particles to a different location. Two spaced opposed electromagnets, however, could also be used. These electromagnets were energized alternately to produce the first and second magnetic fields that keep the particles in suspension and mix them with the fluid in which they were contained.

A method for the separation of magnetic particles from a fluid is disclosed in US patent number 3,985,649. The particles may be separated from a fluid by bringing the particles into close proximity with a magnet and moved through the liquid along the wall of a container. They may even be moved out of the liquid in this way and can be transported to a second container.

In US-A-4,988,618 patent, a device is described for use with assays wherein multiple small volume samples are tested at the same time. This type of assay can be performed in, for example, microtiter plates. Magnetic microparticles are present in each well of the microtiter plate. The device thus has multiple orifices and the orifices are each surrounded by multiple permanent magnets, preferably four. The resulting structure of magnets and orifices is rigid; the magnets are not intended to be moved and are mounted in a fixed relation with respect to themselves and to the base of the device. All magnetic are aligned and the field orientation of the magnets may be such that all magnets have the same field direction or neighbouring magnets have opposite field directions. The magnets orientation thus results in four spot attraction sites per orifice. The magnets are purely meant for separation purposes. It is disclosed in the patent that the device may further comprise means or agitating the reagents within the containers.

The applicant has already filed an international application WO-A-01/05510 which proposes a solution to improve the mixing. It relates to a method and device, which allows efficient mixing of magnetic or magnetizable particles in a fluid and optionally separation of the particles from said fluid. Use is made of magnetic field of opposite and changing directions. It has been found that, when magnetic or magnetizable particles in a fluid are subjected to these magnetic fields, the particles are, under the influence of the filed, efficiently contacted with the fluid. Such particles normally may tend to form a clot, which can prevent efficient mixing with a fluid. It has been found that, by subjecting the container in which the fluid and the particles are comprised, to magnetic fields of different and changing directions, the particles are efficiently separated from each other and drawn trough the fluid in such a way that a very efficient mixing process occurs. The method allows efficient mixing of particles with even very small fluid volumes. The method of the invention therefore has the advantage that it may save in, for example, washing fluids and may allow the reduction of the volume of fluid needed. Thus, for example in isolation procedures, the method of the invention allows the purification of reagents in high concentrations. Beside, whereas prior art methods can be laborious and time consuming; the method is fast and easy to perform.

Thus, provided with this application is a method of mixing, in one or more container(s), magnetic or (super) paramagnetic particles with a fluid, using more than one magnets, whereby the containers are subjected to magnetic fields with different and changing directions by moving the magnets with respect to the position of the container(s) and/or by moving the containers with respect to the positions of the magnets.

State of the art concerning the concentration step:

Many diagnostic tests are carried out after steps of extracting the target analytes from biological samples, of purifying in order to remove parasitic products which penalize the performance of the test, of concentrating the target analytes in order to increase the amount of analyte per unit of buffer volume, and of dissolving the target analytes in a buffer in order to make them chemically accessible.

In addition, in order to increase the sensitivity and the specificity of a test for demonstrating an analyte, it is sometimes necessary to reduce the volume of the buffer in which the copies of the analyte being sought are found, while at the same time conserving said analyte in its entirety.

Biologists have entirely conventional means for concentrating an analyte, in particular using centrifugation, filtration and/or magnetic sedimentation techniques. These techniques require transfers of solutions and manipulations of the analyte, which lead to an inevitable decrease in the amount of analyte that can be analysed.

For example, in centrifugation and magnetic sedimentation methods, the actual centrifugation or magnetic sedimentation steps may have to be repeated several times, the limit of the number of repetitions being set by the minimum volume of solution which can be easily and reliably handled with a conventional pipette. This minimum volume is of the order of about 10 micro litres. Below this, transporting it in "large" containers such as pipettes, flasks, etc loses liquid, and therefore analyte. In addition, there are problems of evaporation and of adsorption to the walls of the containers during these manipulations.

In the case of a low concentration of analyte in the starting sample, this may cause the complete disappearance of the analyte or a decrease in the amount thereof such that it may become undetectable.

Besides the abovementioned drawbacks, these manipulations are expensive in terms of material and take a lot of time. This remains a constant problem for many industrial applications,

for example the detection of pathogenic micro organisms in a biological specimen or an industrial sample.

The international application, WO-A-02/43865, concerns a method for transporting an analyte present in a sample, a method for concentrating an analyte present in a sample, and a device for implementing said methods. The method for transporting an analyte present in a sample consists in preparing a solution from the sample wherein the analyte is fixed on magnetic particles; introducing this solution in a first container connected via a bottle-neck to a second container; displacing with a magnetic system the analyte fixed on magnetic particles from the first container to the second container via the bottle-neck; the second container being filled with all or part of said solution and/or with another solution.

Here again, the solution is provided to how to concentrate magnetic particles into a small volume of liquid. However the separation is not efficiently treated, especially if the magnet is of a smaller size compared to the first container, and the mixing is even not discussed.

A real need therefore exists for a method and a device for treating analytes bound to magnetic particles while at the same time conserving the amount of analyte present at the start, for example in order to increase the sensitivity and the specificity of diagnostic tests and of any chemical reaction directed towards the analyte, and to overcome the abovementioned drawbacks.

The present invention satisfies this need, and has not only the advantage of overcoming the abovementioned drawbacks, but also many other advantages, which those skilled in the art will not fail to note.

Thus none of the above-described documents presents a process offering the advantages of separating, mixing and concentrating molecules of interest from a liquid sample in only one container. Likewise, they do not propose a container having technical features authorizing the running of such a process. Regardless of the claimed method, it may be either manual or automatic, or operated by an automated device or not.

This is the main goal of the present invention to propose a process for manipulating magnetic particles that are suspended in a fluid, possibly containing a biological entity of interest, the magnetic particles being able to bind the entity of interest, the fluid being contained in a reaction

vessel constituted by a large upper compartment with a funnel shape, an elongate lower compartment with a substantially constant cross-section and a closed base, consisting of:

a) subjecting the magnetic particles to two magnetic fields applied simultaneously to separate all said magnetic particles present in at least the upper compartment of the vessel from the fluid,

b) transferring the separated magnetic particles from the upper compartment to the elongated lower compartment,

c) removing the fluid from the vessel,

d) adding a washing liquid to the lower compartment,

e) subjecting the rest of the fluid to at least two magnetic fields applied successively with different and changing directions to wash all the magnetic particles present in the lower compartment, and

f) concentrating said magnetic particles in said lower compartment.

In a particular configuration of the invention, the two magnetic fields applied simultaneously are generated by at least two magnets, the pole axis of the magnets forming together an angle different from 180°, preferably included between 30 and 150° and more preferably included between 60 and 120°.

In another particular configuration of the invention, the at least two magnetic fields applied successively are generated by at least two magnets, the pole axis of the magnets being parallel one to the other.

In a specific embodiment of the latter particular configuration presented just above, the successive magnetic fields with different and changing directions are applied to the vessel by moving the magnets with respect to the position of the vessel and/or by moving the vessel with respect to the position of the magnets.

Always in a particular configuration of the invention, the at least two magnets, cooperating together and having coaxial magnetic fields, and at least two magnets, cooperating together and having magnetic fields that are not coaxial, are positioned on both opposite sides of the vessel.

In a particular configuration of the invention, the magnets are interdependent with one support.

In another particular configuration of the invention, the magnets intended to the separation and the magnets intended to the mixing are identical.

In one embodiment, the support can be moved in rotation around (to pass from separation to mixing configurations) and longitudinally along (to realize the mixing step) an axle passing through each magnet, the axle being parallel to the moving, defined previously, and perpendicularly to magnet's pole axis.

In a particular configuration of the invention, the magnets intended to the separation and the magnets intended to the mixing are different.

According to any of the former particular configurations of the invention, the lower compartment is constituted by:

- one medium compartment to which the magnetic fields are applied successively for washing the magnetic particles, and
- one bottom compartment in which the magnetic particles are concentrated and to which the magnetic fields are applied successively to bring the particles into contact with the elution buffer.

In one particular configuration, in the main process, above disclosed, between step e) and step f), the following intermediate steps are realized:

e1) transferring the separated and mixed magnetic particles from said medium compartment to said bottom compartment,

e2) removing the washing liquid from the vessel, and

e3) adding an elution buffer to the bottom compartment.

In one particular configuration, in the main process, above disclosed, the following further steps are realized after step f):

g) transferring the magnetic particles from said bottom compartment to the medium compartment or to the upper compartment,

h) removing the elution buffer present in the bottom compartment and containing the entity of interest for further processing.

In a preferential configuration, the volume of the medium compartment is smaller compared to the upper compartment and bigger compared to the bottom compartment.

In a particular configuration, the magnet brings about the transfer of magnetic particles.

According to any of the former particular configurations of the invention, the molecules of interest are constituted by nucleic acids (RNA and/or DNA).

On the one hand, the binding of the nucleic acids is non-specific and realized directly onto the surface of the magnetic particles.

On the other hand, the binding of the nucleic acids is specific and realized directly onto capture probes carried by the surface of the magnetic particles.

The present invention also relates to a device for extracting possible molecules of interest from a fluid to which is added a suspension of magnetic or (super) paramagnetic particles contained in at least one reaction vessel and capable to bind the molecules of interest comprising:

- at least one separating station to capture the magnetic particles present in a large upper compartment of each reaction vessel,
- at least one washing station to mix said magnetic particles present in a medium compartment of each reaction vessel,
- at least one concentrating station to mix said magnetic particles present in a bottom compartment of each reaction vessel, and
- at least one pipetting means to dispense and/or to remove part or all of the fluid, the washing liquid and output buffer that are needed to support the process as above presented.

In one embodiment of the device, the separating station comprises at least two magnets, the pole axis of the magnets forming together an angle different from 180°, preferably included between 60 and 150° and more preferably included between 80 and 120°.

In another embodiment of the device, the washing station comprises at least two magnets, the pole axis of the magnets being parallel one to the other.

The invention also relates to a reaction vessel that can be used in an extracting device, above exposed, comprising:

- a top aperture,
- one upper compartment with a funnel shape,
- one medium compartment with a substantially constant cross-section,
- one bottom compartment with a substantially constant cross-section,
- a closed base, and
- a longitudinal axis defined by the lower compartment.

According to one embodiment of the reaction vessel, each of the opposite walls of the upper compartment, constituting the funnel shape, is perpendicular to the pole axis of the magnets that is present with respect to this wall.

According to another embodiment of the reaction vessel, the opposite walls of the upper compartment form together an angle different from 180°, preferably included between 60 and 150° and more preferably included between 80 and 120°.

According to any of the embodiment of the reaction vessel above described, the volume of the medium compartment is smaller compared to the upper compartment (and bigger compared to the bottom compartment.

Again according to any of the embodiment of the reaction vessel above described, the ratio between the volumes of the medium compartment and the upper compartment or between the volumes of the bottom compartment and the medium compartment is comprised between 1:2 to 1:100, preferably between 1:5 to 1:20, and more preferably 1:10.

The invention also relates to a set of reaction vessels constituted by at least two vessels, preferentially at least five vessels and more preferentially eight vessels, according to any of vessels presented above, said vessels being arranged symmetrically along one line.

According to one embodiment of the set of reaction vessels, it cooperates with at least two tips, preferentially at least five tips and more preferentially eight tips, said tips constituting:

- a first pipetting mean to dispense and/or to remove part or all of the fluid,
- a second pipetting mean to dispense and/or to remove part or all of the wash liquid, and
- a third pipetting mean to dispense and/or to remove part or all of output buffer.

According to one embodiment of the set, the free ends of the tips constituting the first or the second or the third pipetting mean being arranged symmetrically along one line or two lines, the two lines of each arrangement being parallel one to the other.

According to another embodiment of the set, the free ends of:

- the tips constituting the first pipetting mean being arranged symmetrically along one line,
- the tips constituting the second pipetting mean being arranged symmetrically along one or two lines, and
- the tips constituting the third pipetting mean being arranged symmetrically along two lines.

According to one embodiment of the set, the first pipetting mean, the second pipetting mean and the third pipetting mean constitute one unique, two or even three different pipetting means.

With "mixing" in this context is meant that the particles and the fluid are brought in close contact. The word "mixing" thus means contacting in a very efficient manner, such as when particles would be washed or reacted with components present in the fluid. Mixing, in this context, does not necessarily provide a homogeneous mixture after the process is finished. The particles may, when the magnets are removed, segregate to the bottom of the container in which they are comprised or may be held to the wall of the container in a particular location by the magnets. The mixing process can for example be used to wash the particles or to react the particles with a component of the liquid, or to bind a component of the liquid to a reagent coated on the particles. Likewise, the mixing process may result in the elution of a certain component originally present on the particles into the surrounding liquid. The method of the invention is applicable in each of these processes and provides an efficient, rapid and convenient way of contacting magnetic or magnetizable particles with a volume of a certain fluid.

The invention will now be described further, by way of examples, with reference to the accompanying drawings, in which:

- Figure 1 is a perspective view of a set constituted by eight containers according to the invention.
- Figure 2 is a cross-sectional view of the container set according to A-A of Figure 1.
- Figure 3 is a cross-sectional view of the container set according to B-B of Figure 2.
- Figure 4 represents detail C of Figure 3.
- Figure 5 represents a view, which is similar to Figure 2, in the case the vessel filled up with the biological sample is disposed between two magnets, acting simultaneously on the fluid.
- Figure 6 represents a view, which is identical to Figure 5, after the two magnets have attracted all magnetic particles present in the starting sample.
- Figure 7 represents a view, which is similar to Figure 6, when said two magnets are moved in downward direction and transfer the magnetic particles from incubation compartment to wash compartment.
- Figure 8 represents a view, which is similar to Figure 7, when the magnets have reached their final position and all magnetic particles have formed a single pellet.
- Figure 9 is a cross-sectional view of one reaction vessel according to D-D of Figure 5.

- Figure 10 represents a view, which is similar to Figure 8, when the vessel is disposed between two other magnets, and after withdrawal of the liquid part of the biological sample.
- Figure 11 represents a view, which is similar to Figure 10, when the wash liquid is added by way of an oblique tip to avoid splashing.
- Figure 12 represents a view, which is similar to Figure 11, showing the to and fro movement of the magnetic particles inside the wash liquid.
- Figure 13 is a cross-sectional view of one reaction vessel according to E-E of Figure 12 explaining the link between the to and fro movement of the magnetic particles and the relative movement of the set of vessels with respect to magnets.
- Figure 14 represents a view, which is similar to Figure 10, when the vessel is disposed between the same two magnets, but after removal of said wash liquid.
- Figure 15 represents a view, which is similar to Figure 14 concerning the magnets position, when the output or elution buffer is added by way of the tip, disclosed in Figure 11, or by way of another tip.
- Figure 16 represents a view, which is quite similar to Figure 7, when the two other magnets are moved downwardly and transfer the magnetic particles from wash compartment to output compartment.
- Figure 17 represents a view, which is similar to Figure 16, after removal of output buffer present in the wash compartment. At this stage, the output buffer is submitted to a heating system in order to achieve elution of molecule of interest.
- Figure 18 represents a view, which is similar to Figure 13, explaining the to and fro movement of the magnetic particles in the output buffer due to the relative movement of the set of vessels with respect to the magnets.
- Figure 19 represents a view, which is quite similar to Figure 16, but using the two former magnets to move upwardly and transfer the magnetic particles from output compartment to wash compartment where no liquid is present.
- Figure 20 represents a view, which is quite similar to Figure 19, where the upward movement is maintained so that the magnets transfer the magnetic particles from wash compartment to incubation compartment to authorize the removal of the rest of output buffer containing the eluted molecules of interest from the output compartment.

- Figure 21 is a perspective view of a main body of tips' support separated from eight tips, tips that could be connected to tip supports having a complementary shape.
- Figure 22 is a bottom view of a main body to which the tips have been connected.
- Finally Figure 23 shows a graphical presentation of the result of a set of real time PCR reactions where a DNA fragment of the Porcine herpesvirus (PhHV-1) has been amplified after isolating the virus from different samples using the device disclosed in this patent application. Along the horizontal axis different sample numbers are indicated. The vertical axis shows the threshold cycle for each sample when a positive PCR signal is observed. Four different sample types have been used (CSF, plasma, serum and blood). With each sample type 20 individual samples have been used.

1 - Description of the preferred embodiment:

1.1 - Details of the reaction vessel:

The vessel 3 is well disclosed on Figures 1-4. It consists in three compartments:

- a large upper incubation compartment 4 with a funnel shape of the vessel 3,
- an elongated medium wash compartment 5 with a constant cross-section of the vessel 3
- an elongated bottom output compartment 6 with a constant cross-section of the vessel 3

This configuration obliges to have a top aperture 19 where it is possible to introduce/dispense or remove any liquid present inside said vessel 3, but also a closed base 7. Finally, according to Figure 4, the vessel 3 has a longitudinal axis 8.

Now referring more particularly to Figure 1, vessels 3 in a preferential configuration are arranged to constitute a set 23, having a manipulation tongue 24 that facilitates the handling by the users. Positioning means not represented on the Figures, could be added in order to render the installation in an automatic device even more efficient than usual.

The main features are:

- Preferably, the total height of the vessel 3 is 40 mm to facilitate recovery of the output buffer 21 from the output compartment 6 using a standard disposable filter tip, like 22, 25, 26.
- Volume of incubation compartment 4 is 2-6 ml, preferably 4ml. In the later configuration, which is the current design, the dimensions are 20 mm in high, 25 mm in depth, 9 mm in width.

- Volume of wash compartment 5 is 0.1-1 ml, preferably 0.2 ml. In the later configuration, which is the current design, the cylindrical shape has 5 mm as diameter and 8 mm as height.
- Volume of output compartment 6 is 10-100 µl, preferably 20 µl. In the later configuration, which is the current design, the cylinder is 5 mm in height and 2 mm in diameter.

The thickness of the wall of the vessel is between 0.2 mm and 1 mm (current design: 0.5 mm).

Preferably, the reaction vessel is a plastic disposable produced by injection moulding for example using polypropylene. To allow convenient manipulation by an operator, it is recommended to integrate several reaction vessels 3 into a set 23. The applicant has realized a design where a set comprises eight vessels 3. For the same reason it is convenient to integrate the tips 21, 25, 26 with their common base 29 to obtain a single unit where the number of tips equals the number of reaction vessels 3 and where the spacing between tips matches with the position of vessels in a set 23.

Preferably the device uses magnets fabricated from neodymium (NdFeB) with a remnant field of 1.2 Tesla. With the current device, cylindrical magnets of 6 mm diameter and 7 mm length have shown suitable for the magnets 9, 10 13 and 14.

When separating magnetic particles 2 from the liquid 1 in the incubation compartment 4, the spacing between the face of the magnets 9 and 10 and the wall of the reaction vessel is typically 0.5 mm. During the wash movement (F11, Figure 13) the magnets 13 and 14 are translated with respect to the vessel with a typical velocity of 1 cm per second. When passing the vessel, the distance to the wall of the wash compartment 5 is typically 1 mm. During the elution movement (F11, Figure 18) the magnets 13 and 14 are translated with respect to the vessel with a typical velocity of 1 cm per second. When passing the vessel, the distance to the wall of the output compartment 6 is typically 2 mm.

1.2 - Method according to the invention:

In a preferred embodiment, the device disclosed in this application is combined with some standard components such as a suction pump and liquid line for aspirating liquid, a liquid dispenser module and some mechanical means for translating the magnets, the aspiration tip and the dispenser with respect to the reaction vessels. In a preferred embodiment, this device is

useful for isolating nucleic acids from a complex starting biological sample, such as whole blood, blood serum, buffy coat (the *crusta phlogistica* or leukocyte fraction of blood), urine, feces, liquor cerebrospinalis, sperm, saliva, tissues, cell cultures and the like. Nucleic acid as isolated from above-mentioned biological material can also comprise the endogenous nucleic acid from the organism from which the sample is derived and any foreign (viral, fungal, bacterial or parasitic) nucleic acid.

The device is used according to the following procedure:

(a) Add liquid mixture to the reaction vessel

A mixture 1 of the primary sample, a lysis buffer and magnetic particles 2 is added to the reaction vessel 3. The target molecules (nucleic acid, not shown on the Figures) are released from the cells or organisms in the sample and bind to the magnetic particles 2. This process step is defined as "incubation". In this step, all the compartments, constituting said vessel 3, are filled up with the fluid 1, i.e. incubation compartment 4, wash compartment 5 and output compartment 6. Due to the difference in size in between said compartments 4-6, most of said fluid 1 is contained in the incubation compartment 4 of the reaction vessel 3. Typically, the incubation time is about 5 minutes.

(b) Separate particles from the sample

After incubation, the liquid mixture 1 in the vessel 3 is disposed between two magnets 9A or 9B or 9C, etc. and respectively 10A or 10B or 10C, etc.; each one 9 or 10 generating a magnetic field respectively 11 and 12, as disclosed in Figure 5. The configuration is also well defined in Figure 9. As a result, the magnetic particles 2 are collected - as a pellet - at two positions at the sidewall of the incubation compartment 4 corresponding to the location of the magnets (Figure 6). Collection time is typically around 1 minute.

(c) Transfer particles to wash compartment

According to Figure 7, the pellets of magnetic particles 2 are transferred from the incubation compartment 4 to the wash compartment 5 by moving the magnets 9 and 10 in the downward direction according to F2. It is also possible to achieve this movement by moving the vessel 3 in the upward direction according to F1, or to combine the down (F2) and upward (F1) movements of respectively said magnets 9 and 10 and said vessel 3. During that

movement(s), the particles 2 initially collected by magnet 10, jump over towards magnet 9, according to F3, where they join with the particles 2 initially collected by magnet 9. After this consolidation, all magnetic particles 2 are transferred to the wash compartment 5 as shown in Figure 8.

(d) Wash magnetic particles

The magnetic particles 2 are washed to remove all sample components as well as other reagents that could interfere with the downstream application. Washing is achieved by disposing the reaction vessel 3 to the magnets 13 and 14 and subsequent removal (F5) of the liquid sample from the reaction vessel 3 using the tip 22 (Figure 10), which is introduced vertically, according to F4, in said vessel 3. Next, according to Figure 11, a fresh wash liquid 20 is added into said vessel 3 according to F9 by mean of a tip 25 introduced in the vessel according to F8, which is at an oblique angle compared to the vertical position of the vessel 3 in order to eliminate any risk of splashes that could generate drops on the internal sidewall, prejudicial to further future process steps. During sample removal and liquid addition, the magnetic particles 2 are retained at the sidewall of the wash compartment 5 under the action of the magnets 13 and 14. Next, the magnets 13 and 14 are moved in the direction F11, as depicted on Figure 13, to transfer the particles 2 back and forth, according to F10 of Figure 12, between opposite sides of the wash compartment 5 to bring them in contact with the wash liquid 20. In those circumstances, magnetic particles 2 are alternatively submitted to two opposite magnetic fields 15 and 16.

Obviously it is also possible to achieve this movement in the F11 direction by moving the vessel 3 with respect to the magnets 13 and 14, or to combine movements of both said magnets 13 and 14 and said vessel 3.

Step (d) can be repeated until sufficient wash performance has been obtained. If appropriate a sequence of different wash liquids can be used. In case different wash liquids are used it will be clear that the dispense tip is washed adequately when changing buffers.

The magnets 13 and 14 are placed in intervening array geometries. This layout allows the use of the method of the invention to give a high throughput format. An embodiment wherein the vessels and the magnets are placed in intervening array geometries is illustrated in Figure 13. The vessels 3 are placed in array geometry with the magnets 13A, 13B, 13C, etc., on one side and 14A,

14B, 14C, etc., on the other side, fixed to a second array 18, used as a support for the magnets, that translates with respect to the vessels 3.

In this way a large series of samples is processed simultaneously. Addition and aspiration of liquids may be by hand or by an automated multi-tip dispenser instrument as know in the art. Such a configuration is well exposed by the Applicant in a former application filed under application number WO-A-01/05510 published the 25th of January 2001 and entitled: "Device and method for mixing magnetic particles with a fluid and optionally separating the particles from the fluid and use thereof in purification methods". Readers could find relevant information about said configuration in this document. This is the reason why its content is enclosed for reference.

(e) Prepare for elution

While the magnetic particles 2 are retained at the sidewall of the wash compartment 5, all wash liquid is removed from the vessel 3, according to F13, using tip 26 that is introduced in the vessel 3 according to F12, to end up in the configuration as depicted in Figure 14. Next, an output or elution buffer 21 is added by way of tip 26 or a new tip, not represented on the drawing, introduced according to F14, to the reaction vessel 3 to fill (F15) the output- and the wash compartments 6 and 5. After that, the magnetic particles 2 are transferred from the wash compartment 5 to the output compartment 6 by moving the magnets 13 and 14 in the downward direction (F16) and/or by moving the vessels 3 in the upward direction (F17) (Figure 16). Next, the volume of output buffer 21 is set by descending tip 26 into the reaction vessel 3, according to F18, while aspirating part of the liquid, according to F19, to a level that corresponds with the specified volume of output buffer (Figure 17).

Although the magnetic particles 2 are in contact with the elution buffer 21 no significant release of target takes place due to the fact that:

- the contact time is short,
- said magnetic particles 2 are collected as a clump, and
- the elution buffer 21 is at room temperature.

(f) Heat the elution buffer

A heater element 33 is enclosing the output compartment 6 of the reaction vessel 3 to heat the output buffer to a specified temperature (Figure 17). A typical temperature for the elution process is 60-80°C. This temperature is applied during about 15 seconds to 10 minutes,

preferably 1 minute to 6 minutes and more preferably 3 minutes. These conditions authorize significant release of target.

(g) Elute nucleic acid from magnetic particles

The target molecules are released from the magnetic particles 2 and recovered in the output buffer 21, only present in the bottom compartment 6, by moving the magnets 13 and 14 in the direction F11, according to Figure 18. Similar to the washing step (d), the particles 2 are translated back and forth between opposite sides of the output compartment 6 to bring said particles 2 in efficient contact with the output buffer 21. During this step the heater 33 is enclosing the output compartment 6 to control the temperature of the output buffer 21.

(h) Separate magnetic particles from output buffer

After the elution step, the magnetic particles 2 are cleared (separated) from the output buffer 21 by disposing the sample vessel 3 to the magnets 9 and 10, see Figure 19, to allow magnet 9 to collect the particles 2 at the sidewall of the output compartment 6 and subsequently move the vessel 3 in the downward direction, according to F20, and/or move the magnets 9 and 10 in the upward direction, according to F21, in order to maintain said particles 2 at the sidewall of the incubation compartment 4, as disclosed in Figure 20, or optionally of the wash compartment 5.

Then the elution buffer 21 present in the output compartment 6 could be used for further processing or, according to Figure 20, be transferred by mean of tip 26, which is introduced (F22) into the vessel 3 and the elution buffer 21, containing the target molecules, is removed by aspiration (F23).

It will be clear to anybody skilled in the art that the device disclosed above as well as the method for using the device is easily integrated in an automated system by performing the translations for the reaction vessel, the magnets, the heater and the tips in an automated way, for example using a set of linear actuators controlled by stepper motors. It is also obvious that, depending on the particular application, particular steps of the above protocol can be omitted such as (f), (g) or (h).

Figures 10 and 14 are quite similar, as the magnetic particles 2 are maintained in position in a single vessel by a only one magnet 13, for instance. Each neighbouring vessel 3 of the set 23 contains also magnetic particles 2 that are attracted by magnet 14. In other words, neighbouring vessels 3 have their magnetic particles 2 on opposite sidewalls. To facilitate the introduction of tips 22 or 25 in the eight vessel's set 23, the positioning of said eight tips has been implemented in an improved manner, as disclosed in Figures 21 and 22. Thus all the tips that collaborate with one set 23 of vessels 3 are associated with a main body 29, this latter having eight tip supports 30, each 30 shifted one to the other. More specifically, each tip comprises two free ends, one is the lower end 27 and the other one is the upper end 28 of the tip. The free ends 27 or 28 are arranged symmetrically along two lines, each line being drawn by four lower end 27 or four upper end 28, and the two lines being parallel one to the other.

Obviously when the tips 22 or 25 are introduced in the vessels 3, according to Figures 10 and 14, the tips are taken away as much as possible from the magnetic particles 2, to avoid any contact prejudicial to further future process steps.

Now referring more particularly to Figure 21, main body 29 in a preferential configuration comprises a manipulation tongue 32 that facilitates the handling by the users. Positioning means not specifically represented on the Figures, could be added in order to render the installation in an automatic device even more efficient than usual. Moreover, said main body 29 comprises an admission/exhaust aperture 31 to permit the dispensing or the removing of any fluid. This aperture 31 could also serve as positioning means.

Four examples are presented where nucleic acids are isolated from an input sample using the invention presented above. With these examples the wash buffers, the elution buffer and the magnetic particles were obtained from the "*NucliSens magnetic extraction reagents*" (article code 200297). The lysis buffer is the "*NucliSens Lysis buffer*"(article code 200295). NucliSens products are supplied by bioMérieux B. V. (Boxtel, The Netherlands)

2 – Example 1: Concentration of ribosomal RNA from plasma samples:

2.1 - Materials and methods:

Target RNA is extracted from *Escherichia coli* cells using the Qiagen RNA/DNA maxi kit (article code 14162, Qiagen, Hilden, Germany). For each sample, 2 micrograms (µg) are used as input.

Sample is constituted by normal EDTA/citrate plasma samples from a pool of 100 individual blood donations.

The RNA quantification is performed using a luminescent marker for RNA (Sybr Green-II, supplied by Molecular Probes: article S-7564) in combination with a luminescent reader for microtiter plates ("Victor^2", supplier Wallac Oy, Turku, Finland: 1420-multilabel counter).

Purity of the output buffer is determined from the relative value for the spectral absorption at 260nm and 280nm (A260/A280) using an UV spectrophotometer (supplier Unicam, Cambridge, Great Britain: Unicam UV-1).

2.2 - Sample preparation:

A set of twenty-four plasma samples is prepared as follows:

- With each sample, 1 ml of plasma is added to the reaction vessel and mixed with 2 ml NucliSens Lysis buffer.
- Next, the target rRNA is added to that mixture using an input of 2 µg RNA for each sample.
- Subsequently, 1 mg of magnetic particles is added to the lysed plasma samples and a homogeneous dispersion is produced using a manual pipette.

2.3 - Extraction method:

A batch of twenty-four samples is processed simultaneously according to the procedure indicated above as steps (a) to (g). The samples are added to three sets of reaction vessels, each set comprising eight identical vessels.

After collecting the magnetic particles from the incubation compartment (collection time was 1 minute), the particles are transferred to the wash compartment and washed using NucliSens wash buffer 1 and wash buffer 2, using two cycles of 3 ml and 1 ml in the first and second cycle respectively.

After washing, the particles are transferred to the output compartment where the target RNA is released from the particles by mixing the particles in the NucliSens elution buffer

(wash buffer 3) for 5 minutes. During elution, the temperature of the buffer is 60°C. The volume of the output buffer is 20 µl.

After elution, the magnetic particles are separated from the output buffer using the magnets 9.

After completing the protocol, the amount of RNA recovered in the output compartment of each vessel is measured using a luminescent RNA marker. For that purpose, 10 µl of the output buffer is transferred from each reaction vessel into the well of a microtiter plate and 190 µl of Sybr Green solution is added to each well. In each well, the optical signal detected by the luminescent plate reader (Victor^2 is a direct measure for the amount of RNA that is present in that well.

2.4 - Result:

For the twenty-four samples, the average amount of target rRNA in the output compartment of the vessel is 1,32 µg with a standard deviation of 50 ng. This corresponds to an average yield of 66%.

Purity of the output is excellent as concluded from the A260/A280 ratio using the UV spectrophotometer. The output buffer shows a ratio of 2.1 whereas pure RNA in this buffer has an A260/A280 ratio between 1.9 and 2.1.

Consistency of the target RNA is determined using the 2100 Bioanalyzer (Agilent Technologies, Amstelveen, the Netherlands). This instrument detects the two discrete RNA bands corresponding to the 16S and 23S RNA. No other products such as smaller RNA fragments have been detected.

The total time for completing the extraction procedure for this batch of twenty-four samples is 30 minutes, starting after adding the magnetic particles to the lysed samples. No intervention from an operator is needed to produce the concentrated RNA in the output.

3 - Example 2: Concentration of plasmid DNA from plasma samples:

3.1 - Materials and methods:

Target is a plasmid DNA, pBR322 (article N3033L, New England Biolabs Inc., Beverly, MA), linearized by BamH1 (article E1010WH, Amersham Bioscience Corp, NY, USA) . Six µg of DNA is used as input per sample

The sample is constituted by 0.1ml normal EDTA/citrate plasma from blood donations

3.2 - Sample preparation:

Twenty-four samples are processed simultaneously, using the same procedure as with example 1. The individual plasma samples are added to the reaction vessels. Next, 2 ml of NucliSens lysis buffer are added. Subsequently, the plasmid DNA is spiked to the sample and 1 mg of magnetic particles is added to the mixture.

3.3 - Extraction method:

The collection step, washing steps and elution step of the extraction procedure are identical to example 1.

3.4 – Result:

After completing the procedure, the amount of extracted DNA is determined from the optical density of the output buffer at 260 nm.

The average amount of DNA recovered from the reaction vessel is 3.1 µg with a variation coefficient of 4 % between different vessels. This corresponds to an isolation yield of 52 % using 6 µg of DNA as input.

The average A260/A280 ratio for the output buffer is 1.9. For pure DNA in the output buffer this ratio is between 1.7 and 2.1.

As with example 1, the time needed to complete the extraction procedure is 30 minutes starting after adding the magnetic particles.

4 - Example 3: Recovery of viral RNA from sputum samples:

4.1 - Materials and methods:

In this experiment, the recovery of HIV RNA that is spiked to nine sputum samples is determined using the NucliSens EasyQ HIV-1 assay version 1.1 (article 285029, bioMérieux B.V., Boxtel, The Netherlands).

The RNA is HIV RNA obtained from a cultured HIV-1 type B virus stock (HXB2) that is lysed using the NucliSens lysis buffer and calibrated against the WHO International HIV-1 RNA standard.

The sputum samples were obtained from the University Hospital of Antwerpen (Belgium).

4.2 – Sample preparation:

With each sputum sample a volume of 1 ml is mixed with 0.5 ml of protease solution (100 mg/ml) and incubated on a plate shaker for 20 minutes at room temperature to obtain a liquefied sample.

The target HIV RNA is spiked to a tube containing 2 ml of NucliSens lysis buffer, using 30.000 copies input for each sample. Together with the HIV RNA, a calibrator RNA that is part of the EasyQ kit is added to the lysis buffer. Next the liquefied sputum samples are added to the reaction vessel together with the lysis buffer (2 ml) containing the RNA. After 10 minutes, 1 mg of magnetic particles is added to each sample. A homogeneous dispersion is obtained by mixing with a manual pipette.

4.3 - RNA extraction:

The extraction procedure proceeds as with the examples 1 and 2 described above.

4.4 - RNA detection:

At the end of the extraction procedure, the HIV RNA and calibrator RNA are concentrated in 12 µl of elution buffer in the output compartment. Two fractions of 5 µl each are aspirated from the output compartment and used as input in a NucliSens EasyQ assay. For the detection, the procedure as indicated in the EasyQ assay manual is followed.

4.5 - Result:

The results are presented in the table here below:

Sample number	EasyQ result (viral copies)	
	Reaction 1	Reaction 2
1	11.000	10.000
2	11.000	9.700
3	9.600	9.200
4	12.000	13.000

5	11.000	16.000
6	18.000	13.000
7	9.200	10.000
8	12.000	14.000
9	20.000	12.000

From these results, we conclude that the device disclosed in this application is well able to isolate viral RNA from sputum samples and concentrate it in a form that facilitates detection using standard amplification methods.

5 - Experiment 4: Recovery of viral DNA from different sample types:

5.1 - Materials and methods:

This experiment represents a series of four extraction runs on twenty-two samples each. With each run a different sample type is used:

- Run 1: plasma samples from individual donations (1 ml per sample)
- Run 2: Cerebra Spinal Fluid (CSF) (0.1 ml per sample)
- Run 3: serum samples from individual donations using 1 ml per sample
- Run 4: whole blood samples (0.1 ml per sample).

5.2 – Sample preparation:

Each sample is added to 2 ml NucliSens lysis buffer.

As a target, the Porcine herpesvirus (PhHV-1) is spiked to twenty of the lysed samples in each run. Two samples (21st and 22nd) were used as a negative control (no DNA spiked).

5.3 - DNA extraction:

The extraction method for each run (sample type) is identical to the procedure described in the examples 1, 2 and 3.

5.4 - DNA detection:

The DNA that is recovered in the output buffer is detected using a real time PCR. The number of PCR cycles (CT) needed to produce a positive signal is determined for each sample.

The figure 23 shows a graphical presentation of these CT values for all samples. The negative control samples were all negative.

5.5 - Result:

From this set of experiments, we conclude that the device disclosed in this patent application is well able to isolate nucleic acid from virus particles from different sample types with excellent yield.

REFERENCES

1. Fluid or mixture
2. Magnetic particles
3. Reaction vessel
4. Large upper incubation compartment with a funnel shape of the vessel 3
5. Elongate medium wash compartment with a constant cross-section of the vessel 3
6. Elongate bottom output compartment with a constant cross-section of the vessel 3
7. Closed base of the vessel 3
8. Longitudinal axis of the vessel 3
9. Magnets (A, B, C, D, etc.) present on one side of the container set used for the separation step
10. Magnets (A, B, C, D, etc.) present on the other side of the container set used for the separation step
11. Pole axis (A, B, C, D, etc.) of the magnet (9A, 9B, 9C, 9D, etc.)
12. Pole axis (A, B, C, D, etc.) of the magnet (10A, 10B, 10C, 10D, etc.)
13. Magnets (A, B, etc.) present on one side of the container set used for the mixing and/or the concentrating step(s)
14. Magnets (A, B, etc.) present on the other side of the container set used for the mixing and/or the concentrating step(s)
15. Pole axis (A, B, etc.) of the magnet (13A, 13B, 13C, 13D, etc.)
16. Pole axis (A, B, etc.) of the magnet (14A, 14B, 14C, 14D, etc.)
17. Support of the magnets (9A, 9B, 9C, 9D, etc., or 10A, 10B, 10C, 10D, etc.)
18. Support of the magnets (13A, 13B, 13C, 13D, etc., or 14A, 14B, 14C, 14D, etc.)

19. Top aperture of the vessel 3
20. Wash liquid
21. Output buffer
22. Tip for adding or removing fluid 1
23. Set of vessels 3
24. Manipulation tongue of vessels' set 23
25. Tip for adding wash liquid 20 and/or elution buffer 21
26. Tip for removing wash liquid 20 and/or output buffer 21
27. Lower end of tip 25
28. Upper end of tip 25
29. Main body of tips' support 30
30. Tip support
31. Admission/exhaust aperture of the main body 29
32. Manipulation tongue of main body 29
33. Heating system

F1. Upward movement of vessel 3
F2. Downward movement of magnets 9 and 10
F3. Magnetic attraction of particles 2
F4. Downward movement of tip 22
F5. Removing of fluid 1 by aspiration with tip 22
F8. Downward movement of tip 25
F9. Dispensing wash liquid 20 expulsed from tip 25
F10. Movement to and fro of magnetic particles 2
F11. Relative movement of the set 23 with respect to magnets 13 and 14
F12. Downward movement of tip 25
F13. Removing of wash liquid 20 by aspiration with tip 25
F14. Downward movement of tip 26
F15. Dispensing output buffer 21 expulsed from tip 26
F16. Downward movement of magnets 13 and 14
F17. Upward movement of vessel 3
F18. Downward movement of tip 26
F19. Removing output buffer 21 present in wash compartment 5

F20. Downward movement of vessel 3

F21. Upward movement of the magnets 13 and 14

F22. Downward movement of tip 26

F23. Removing of output buffer 21 by aspiration with tip 26

5

CLAIMS

1. A process for manipulating magnetic particles (2) that are suspended in a fluid (1), possibly containing a biological entity of interest, the magnetic particles (2) being able to bind the entity of interest, the fluid (1) being contained in a reaction vessel (3) constituted by a large upper compartment with a funnel shape (4), an elongate lower compartment with a substantially constant cross-section (5 and 6) and a closed base (7), consisting of:

a) subjecting the magnetic particles (2) to two magnetic fields applied simultaneously to separate all said magnetic particles (2) present in at least the upper compartment (4) of the vessel (3) from the fluid (1),

b) transferring the separated magnetic particles (2) from the upper compartment (4) to the elongated lower compartment (5 and 6),

c) removing the fluid (1) from the vessel (3),

d) adding a washing liquid (20) to the lower compartment (5 and 6),

e) subjecting the rest of the fluid (1) to at least two magnetic fields applied successively with different and changing directions to wash all the magnetic particles (2) present in the lower compartment (5 and 6), and

f) concentrating said magnetic particles (2) in said lower compartment (5 and 6).

2. A process, according to claim 1, wherein the two magnetic fields applied simultaneously are generated by at least two magnets (9A and 10A), the pole axis (11A or 12A) of the magnets (9A, 10A) forming together an angle different from 180°, preferably included between 30 and 150° and more preferably included between 60 and 120°.

3. A process, according to any of claims 1 or 2, wherein the at least two magnetic fields applied successively are generated by at least two magnets (13A and 14A), the pole axis (15A or 16A) of the magnets (13A, 14A) being parallel one to the other.

4. A process, according to claim 3, wherein the successive magnetic fields with different and changing directions are applied to the vessel (3) by moving the magnets (13A and 14A) with respect to the position of the vessel (3) and/or by moving the vessel (3) with respect to the position of the magnets (13A and 14A).

5. A process, according to any of claims 2-4, wherein the at least two magnets (9A and 13A) and at least two magnets (10A and 14A) are positioned on both opposite sides of the vessel (3).

6. A process, according to any of claims 2-5, wherein the magnets (9A, 10A, 13A, 14A) are interdependent with one support.

7. A process, according to any of claims 2-6, wherein the magnets intended to the separation and the magnets intended to the mixing are identical.

8. A process, according to the combination of both claims 6 and 7, wherein the support can be moved in rotation around (to pass from separation to mixing configurations) and longitudinally along (to realize the mixing step) an axle passing through each magnet, the axle being parallel to the moving, defined in claim 4, and perpendicularly to magnet's pole axis.

9. A process, according to any of claims 2-6, wherein the magnets (9A and 10A) intended to the separation and the magnets (13A and 14A) intended to the mixing are different.

10. A process, according to any of claim 1-9, wherein the lower compartment is constituted by:

- one medium compartment (5) to which the magnetic fields are applied successively, and
- one bottom compartment (6) in which the magnetic particles (2) are concentrated.

11. A process, according to claim 10, wherein between step e) and step f) exposed in claim 1 the following intermediate steps are realized:

e1) transferring the separated and mixed magnetic particles (2) from said medium compartment (5) to said bottom compartment (6),

e2) removing the washing liquid (20) from the vessel (3), and

e3) adding an elution buffer (21) to the bottom compartment (6).

12. A process, according to any of claims 10 or 11, wherein the following further steps are realized after step f) of claim 1:

g) transferring the magnetic particles (2) from said bottom compartment (6) to the medium compartment (5) or to the upper compartment (4),

h) removing the elution buffer (21) present in the bottom compartment (6) and containing the entity of interest for further processing.

13. A process, according to any of claims 10-12, wherein the volume of the medium compartment (5) is smaller compared to the upper compartment (4) and bigger compared to the bottom compartment (6).

14. A process, according to any of claims 1 or 10-13, wherein the transfer of magnetic particles (2) is sustained by the magnet (9A).

15. A process, according to any of claims 1-14, wherein the molecules of interest are constituted by nucleic acids (RNA and/or DNA).

16. A process, according to claim 15, wherein the binding of the nucleic acids is non-specific and realized directly onto the surface of the magnetic particles (2).

17. A process, according to claims 15, wherein the binding of the nucleic acids is specific and realized directly onto capture probes carried by the surface of the magnetic particles (2).

18. Device for extracting possible molecules of interest from a fluid (1) to which is added a suspension of magnetic or (super) paramagnetic particles (2) contained in at least one reaction vessel (3) and capable to bind the molecules of interest comprising:

- at least one separating station to capture the magnetic particles (2) present in a large upper compartment (4) of each reaction vessel (3),
- at least one washing station to mix said magnetic particles (2) present in a medium compartment (5) of each reaction vessel (3),
- at least one concentrating station to mix said magnetic particles (2) present in a bottom compartment (6) of each reaction vessel (3), and

- at least one pipetting means to dispense and/or to remove part or all of the fluid (1), the washing liquid (20) and output buffer (21) that are needed to support the process according to claims 1-17.

19. Device, according to claim 18, characterized in that the separating station comprises at least two magnets (9A and 10A), the pole axis (11A and 12A) of the magnets (9A and 10A) forming together an angle different from 180°, preferably included between 60 and 150° and more preferably included between 80 and 120°.

20. Device, according to claim 18, characterized in that the washing station comprises at least two magnets (13A and 14A), the pole axis (15A and 16A) of the magnets (13A and 14A) being parallel one to the other.

21. Reaction vessel (3) that can be used in an extracting device according to any of claims 18-20 comprising:

- a top aperture (19),
- one upper compartment (4) with a funnel shape,
- one medium compartment (5) with a substantially constant cross-section,
- one bottom compartment (6) with a substantially constant cross-section,
- a closed base (7), and
- a longitudinal axis (6) defined by the lower compartment (5 and 6).

22. Reaction vessel, according to claim 21, characterized in that each of the opposite walls of the upper compartment (4), constituting the funnel shape, is perpendicular to the pole axis (11A and 12A) of the magnets (9A and 10A) that is present with respect to this wall.

23. Reaction vessel, according to claim 22, characterized in that the opposite walls of the upper compartment (4) form together an angle different from 180°, preferably included between 60 and 150° and more preferably included between 80 and 120°.

24. Reaction vessel, according to any of claims 21-23, wherein the volume of the medium compartment (5) is smaller compared to the upper compartment (4) and bigger compared to the bottom compartment (6).

25. Reaction vessel, according to any of claims 21-24, wherein the ratio between the volumes of the medium compartment (5) and the upper compartment (4) or between the volumes of the bottom compartment (6) and the medium compartment (5) is comprised between 1:2 to 1:100, preferably between 1:5 to 1:20 and more preferably between 1:10.

26. Set (23) of reaction vessels (3) constituted by at least two vessels (3), preferentially at least five vessels (3) and more preferentially eight vessels (3), according to any of claims 21-25, said vessels (3) being arranged symmetrically along one line.

27. Set of reaction vessels, according to claim 26, wherein it cooperates with at least two tips, preferentially at least five tips and more preferentially eight tips, said tips constituting:

- a first pipetting mean to dispense and/or to remove part or all of the fluid (1),
- a second pipetting mean to dispense and/or to remove part or all of the washing liquid (20), and
- a third pipetting mean to dispense and/or to remove part or all of output buffer (21).

28. Set of reaction vessels, according to claim 27, wherein the free ends of the tips constituting the first or the second or the third pipetting mean being arranged symmetrically along one line or two lines, the two lines of each arrangement being parallel one to the other.

29. Set of reaction vessels, according to claim 28, wherein the free ends of:

- the tips constituting the first pipetting mean being arranged symmetrically along one line,
- the tips constituting the second pipetting mean being arranged symmetrically along one or two lines, and
- the tips constituting the third pipetting mean being arranged symmetrically along two lines.

30. Set of reaction vessels, according to any of claims 28-29, wherein the first pipetting mean, the second pipetting mean and the third pipetting mean constitute one unique, two or even three different pipetting means.

5

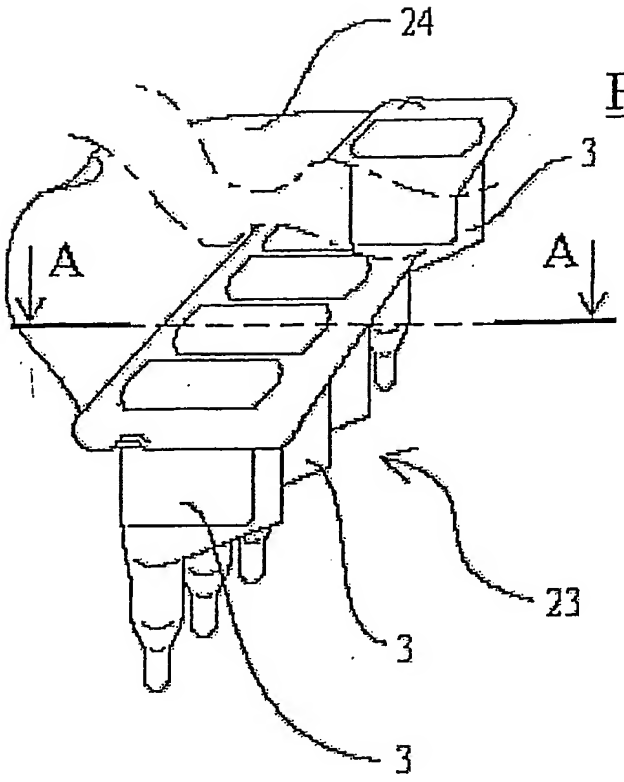
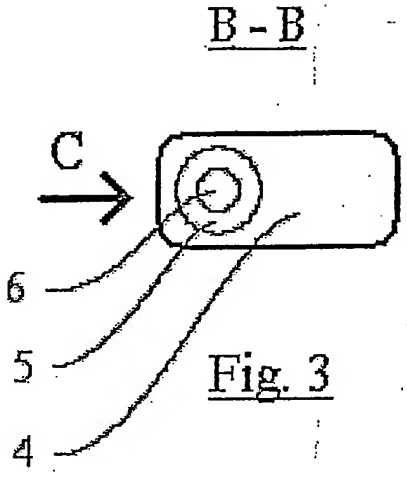


Fig. 1



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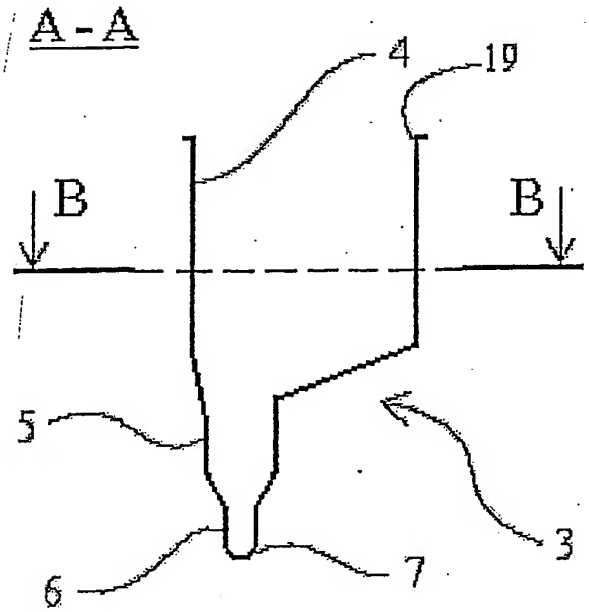
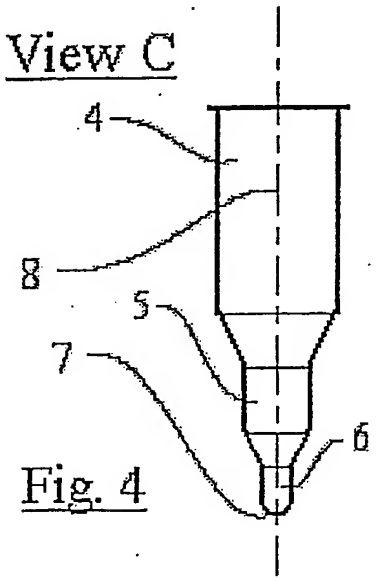


Fig. 2



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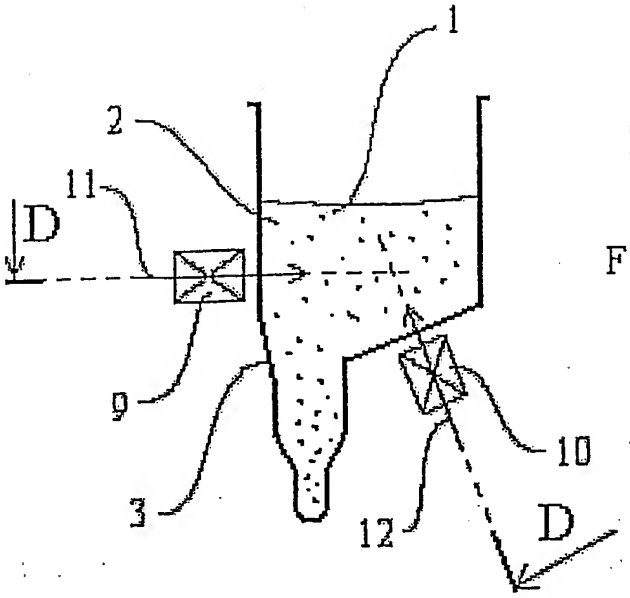

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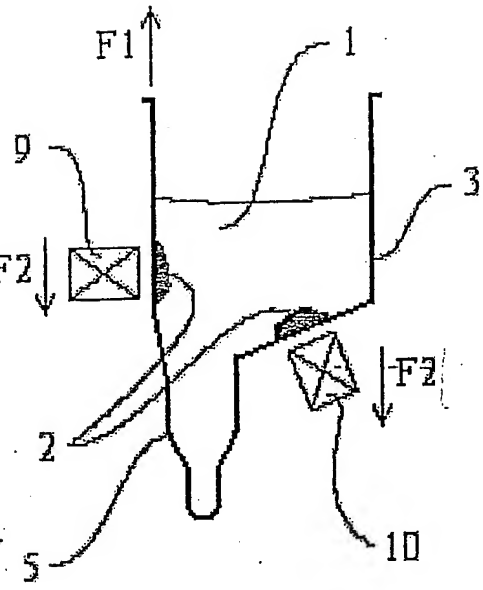


Fig. 6

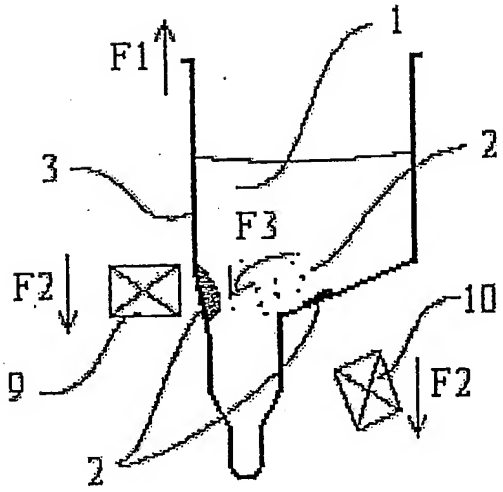


Fig. 7

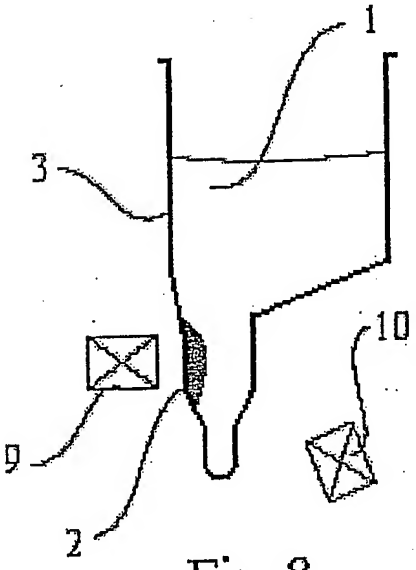


Fig. 8

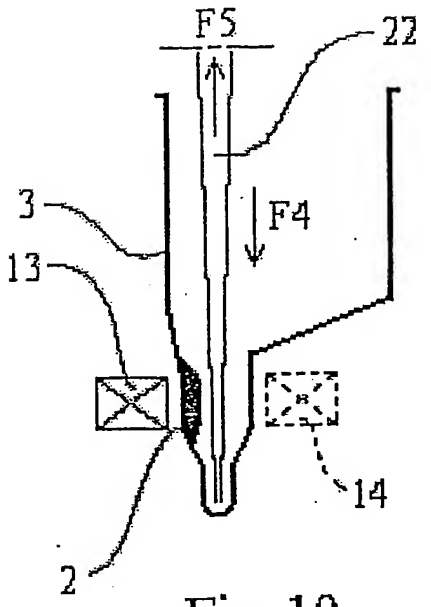


Fig. 10

D - D

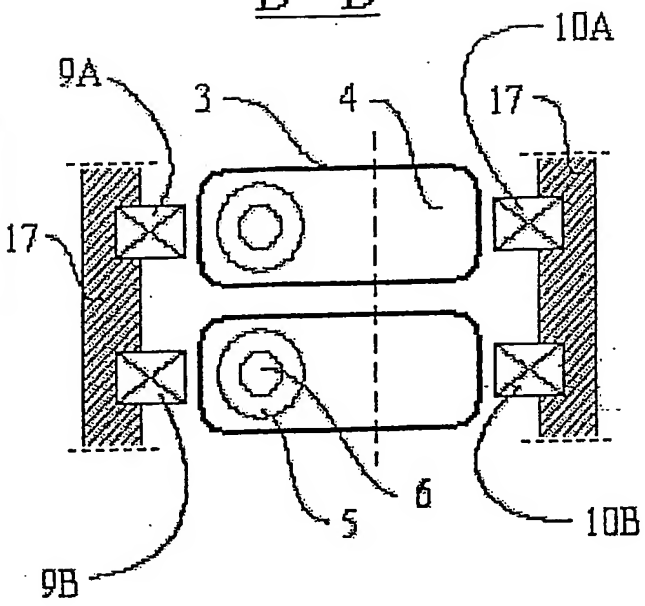


Fig. 9

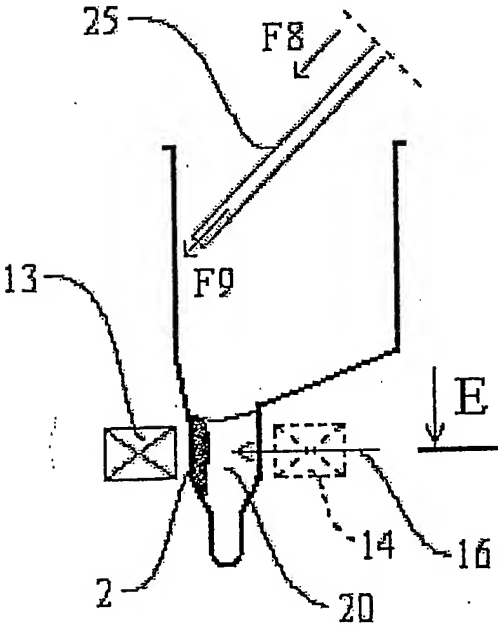


Fig. 11

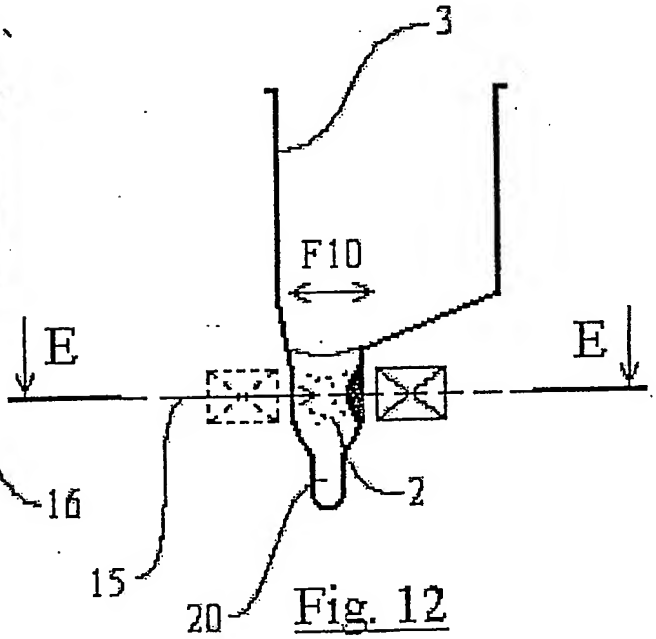


Fig. 12

E - E

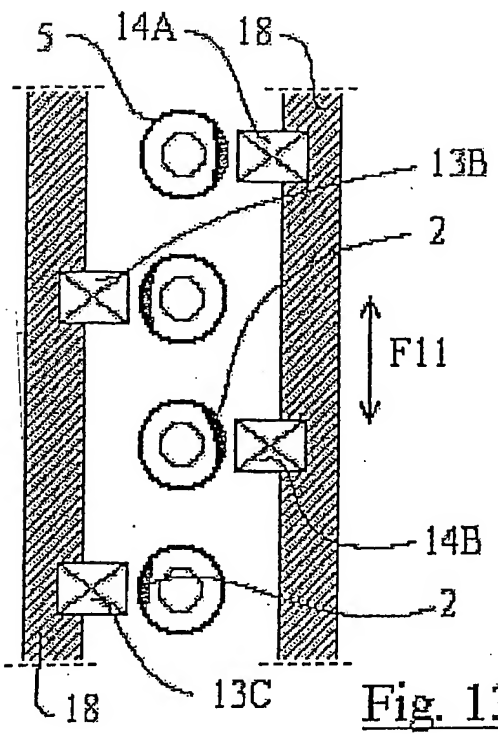


Fig. 13

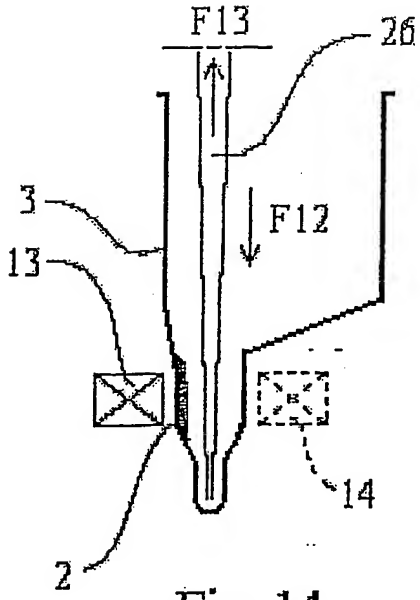


Fig. 14

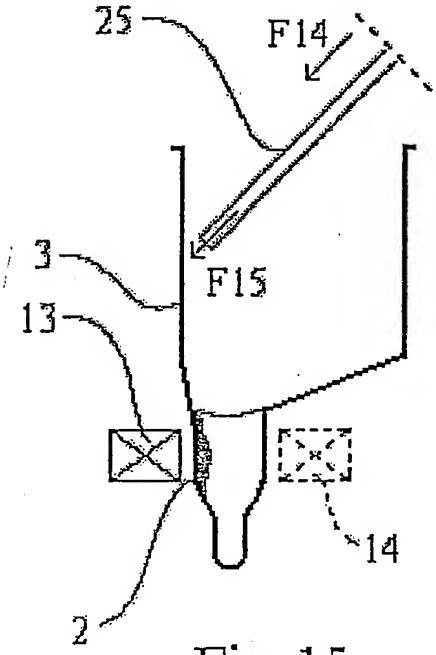


Fig. 15

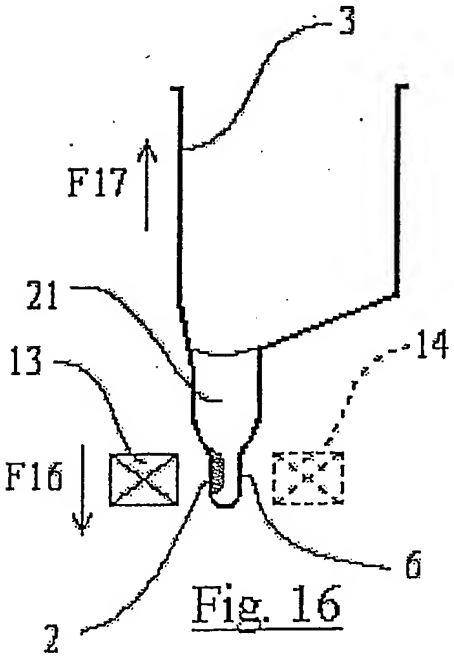


Fig. 16

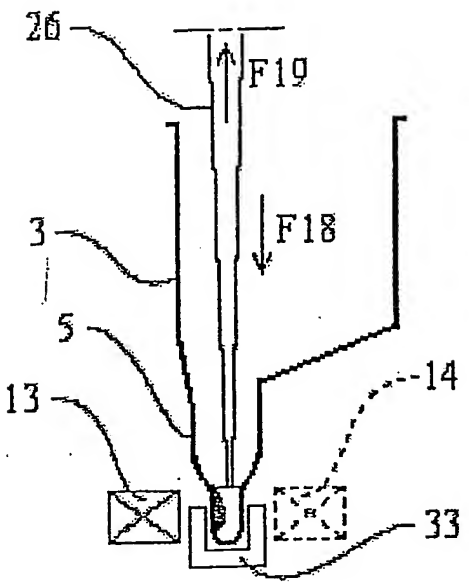


Fig. 17

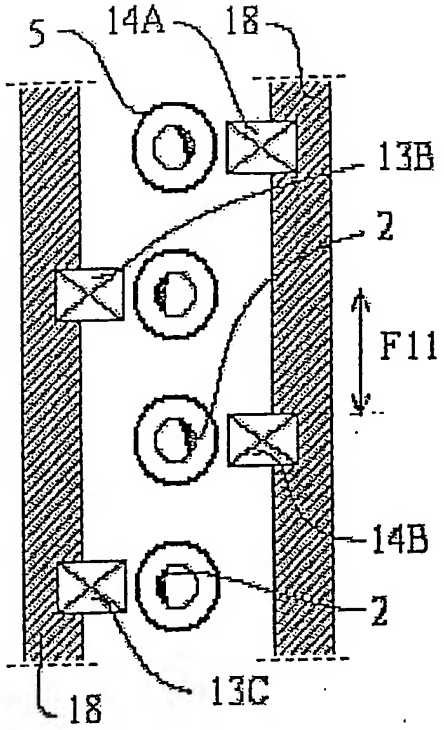
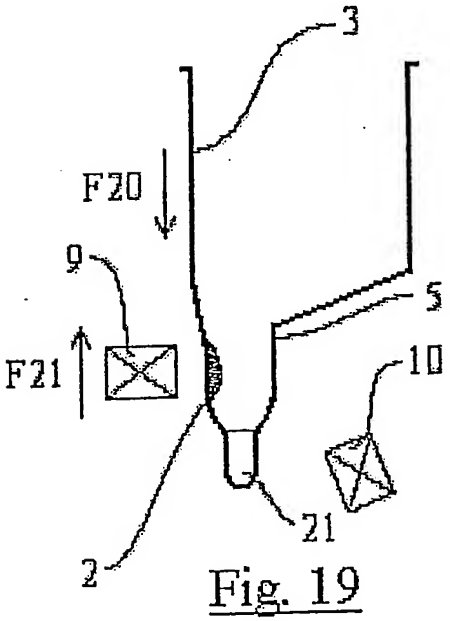


Fig. 18



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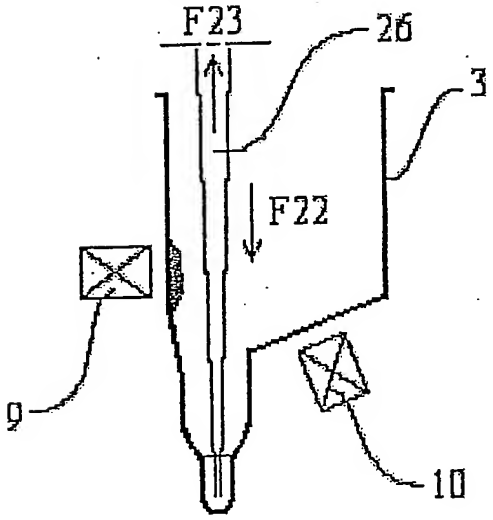

Fig. 20

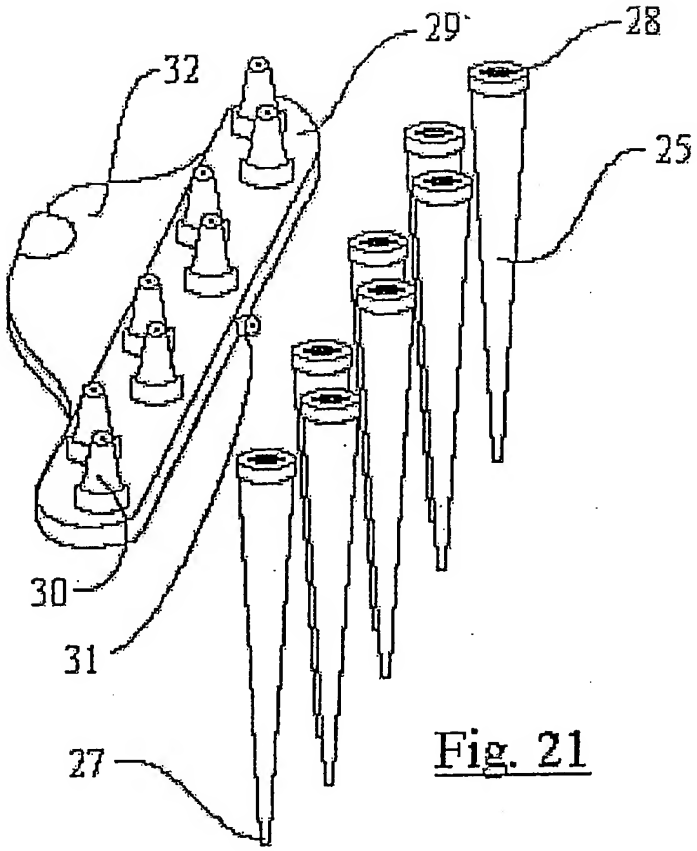

Fig. 21

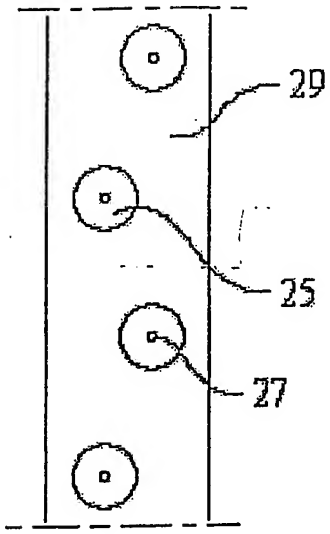

Fig. 22

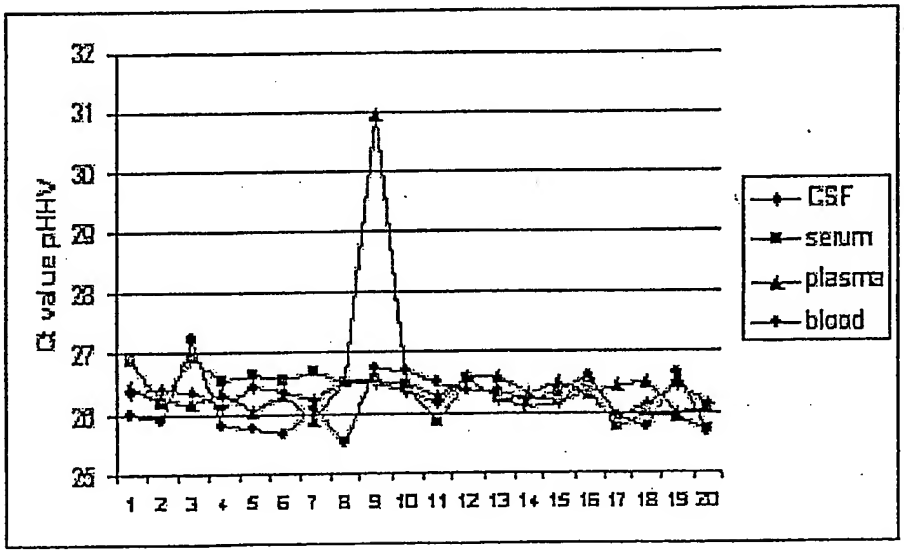

Fig. 23

PCT/EP2005/008073

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N35/00 B01L3/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01L C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/05510 A (VERWIMP EMIEL GEREBERN MARIA ; AKZO NOBEL NV (NL); KREUWEL HERMANUS JO) 25 January 2001 (2001-01-25) cited in the application the whole document figure 4	1-30
A	WO 03/006168 A (BIOMERIEUX SA ; COLIN BRUNO (FR)) 23 January 2003 (2003-01-23) the whole document figures 1-3	1-30
A	EP 0 317 286 A (GENE TRAK SYSTEMS) 24 May 1989 (1989-05-24) cited in the application the whole document figure 1A	1-30

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2005	28/10/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Petri, B

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 644 425 A (HOFFMANN LA ROCHE) 22 March 1995 (1995-03-22) the whole document figure 1	1-30

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